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The sexual state of *Aspergillus parasiticus*

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Abstract: The sexual state of *Aspergillus parasiticus*, a potent aflatoxin-producing fungus within section *Flavi*, is described. The production of nonostiolate ascocarps surrounded by a separate peridium within the stroma places the teleomorph in genus *Petromyces*. *Petromyces parasiticus* differs from *P. alliaceus* by its larger ascospores with finely tuberculate ornamentation. The anamorphic *Aspergillus* states of the two species differ in conidial head color and microscopic characters.

Key words: *Aspergillus alliaceus*, heterothallism, *Petromyces alliaceus*, *Petromyces parasiticus*, sexual reproduction, Trichocomaceae

INTRODUCTION

A.T. Speare, a pathologist with the Hawaiian Sugar Planters' Association, first described *Aspergillus parasiticus* Speare (1912) from sugarcane plantations on the islands of Kauai and Oahu. He observed the fungus sporulating on sugarcane mealy bugs that had died while feeding on the cane leaf sheaths. Approximately 50 y later *A. parasiticus* along with *A. flavus* Link gained worldwide attention with the discovery of aflatoxins produced by these fungi in crops (Cullen and Newberne 1994). A disease outbreak in ducklings in Kenya due to aflatoxins in Ugandan peanut meal (Asplin and Carnaghan 1961, Sargeant et al 1961) initially was linked to *A. flavus*, but the fungus subsequently was identified as *A. parasiticus* (Cole 1986). Aflatoxins are among the most potent carcinogens known and are also acutely hepatotoxic and immunosuppressive in a variety of animals (Eaton and Groopman 1994, Turner et al 2003). Many countries have strict limits on the amount of aflatoxins permitted in human commodities and animal feed (van Egmond and Jonker 2005).

Aspergillus parasiticus and *A. flavus* belong to section *Flavi* and are closely related yet separable based on DNA sequence and AFLP fingerprint analyses (Barros et al 2007, Peterson 2008). The two species are also easily distinguished phenotypically. *Aspergillus parasiticus* produces aflatoxins B_1, B_2, G_1 and G_2, whereas aflatoxigenic strains of *A. flavus* typically produce only B aflatoxins; another unrelated mycotoxin, cyclopiazonic acid, is produced by *A. flavus* but not *A. parasiticus* (Horn et al 1996). In addition *A. parasiticus* can be separated from *A. flavus* by the darker green conidial heads and by the more pronounced conidium ornamentation (Klich and Pitt 1988).

Populations of *A. parasiticus* are characterized by considerable diversity in morphology and aflatoxin production (Horn et al 1996). Genetic diversity in *A. parasiticus* populations is indicated by the large numbers of vegetative compatibility groups (Horn and Greene 1995), which provide a multilocus measure of diversity, and by the numerous DNA fingerprint groups (McAlpin et al 1998). The high genetic variation in *A. parasiticus* is not easily explained in the absence of sexuality (Horn 2007). Many species in section *Flavi*, including *A. parasiticus*, produce sclerotia (Raper and Fennell 1965, Horn et al 1996). Fennell and Warcup (1959) discovered ascocarps forming within the "sclerotia" of *A. alliaceus* Thom & Church (teleomorph = *Petromyces alliaceus* Malloch & Cain), a species that does not produce aflatoxins, but their concerted effort over many years to induce sexuality in other sclerotium-producing section *Flavi* species proved unsuccessful. Therefore *Aspergillus* species such as *A. parasiticus* and *A. flavus* were considered to be strictly asexual and to have lost their ability to undergo meiosis (Geiser et al 1996).

The possibility of a cryptic sexual stage in aflatoxin-producing Aspergilli first was suggested by Geiser et al (1998), who reported a population structure in *A. flavus* indicative of recombination based on a lack of congruence of five gene trees, though the method of recombination, how often recombination occurs, and when recombination occurred in the history of the species could not be determined. Similar evidence for recombination based on discordance of gene trees for four genes was shown for *A. nomius* Kurtzman et al, another aflatoxin-producing species (Peterson et al 2001). Carbone et al (2007) sequenced 21 intergenic

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regions of the aflatoxin gene cluster in strains from an *A. parasiticus* population in a Georgia peanut field. The population had been characterized previously according to morphology, mycotoxin production and vegetative compatibility (Horn and Greene 1995, Horn et al 1996). Significant linkage disequilibrium in the evolutionary history of the species was detected, and the gene cluster could be divided into five recombination blocks. Furthermore genealogical analysis of haplotype data suggested that some recombination had occurred within the past 1 000 000 y.

Genes *MAT1-1* and *MAT1-2* responsible for mating compatibility were identified in the *A. parasiticus* Georgia population as well as the *A. flavus* population from the same peanut field (Ramirez-Prado et al 2008). Individual strains of *A. parasiticus* and *A. flavus* each contained a single *MAT* gene, indicating that both species are heterothallic. Furthermore gene expression by *MAT1-1* and *MAT1-2* at the mRNA level was detected and the frequency of the two mating types was nearly equal in clone-corrected population samples, providing additional evidence for the presence of a sexual state in nature. Detailed knowledge of the sexual compatibility system led to crosses in culture between *A. parasiticus* strains of the opposite mating type. After an extended incubation period (6–9 mo) ascocarps formed within sclerotia (now considered stromata) in a manner similar to *P. alliaceus* (Horn et al 2009). Recombination through the independent assortment of chromosomes was detected using loci for mating type, aflatoxin gene cluster and a protein-encoding gene.

The teleomorph associated with *A. parasiticus* is formally described in this paper.

MATERIALS AND METHODS

Strains of *A. parasiticus* were obtained from soil and peanut seeds in a peanut field in Terrell County, Georgia, USA (Horn and Greene 1995). *MAT1-1* and *MAT1-2* genes were identified in strains by Ramirez-Prado et al (2008). Strains of the opposite mating type were paired on slants of mixed cereal agar (McAlpin and Wicklow 2005a) and were incubated in darkness at 30 C in sealed plastic bags 6–9 mo (Horn et al 2009). Morphological characters of the *Aspergillus* anamorph were based on growth on Czapek agar and malt extract agar (Raper and Fennell 1965).

Sclerotia and stromata were harvested from culture plates and dissected as described by Horn et al (2009). Measurements at low magnifications were made with a Leica MZ16FA stereomicroscope equipped with a DFC-300FX digital camera. Stacked images obtained with this camera were combined with Image Pro Express v.5.0 software module. For higher magnifications, measurements were made with a Zeiss Photomicroscope I, and differential interference contrast images were obtained with a Nikon Eclipse E600 microscope equipped with a Nikon CoolPix 995 digital camera.

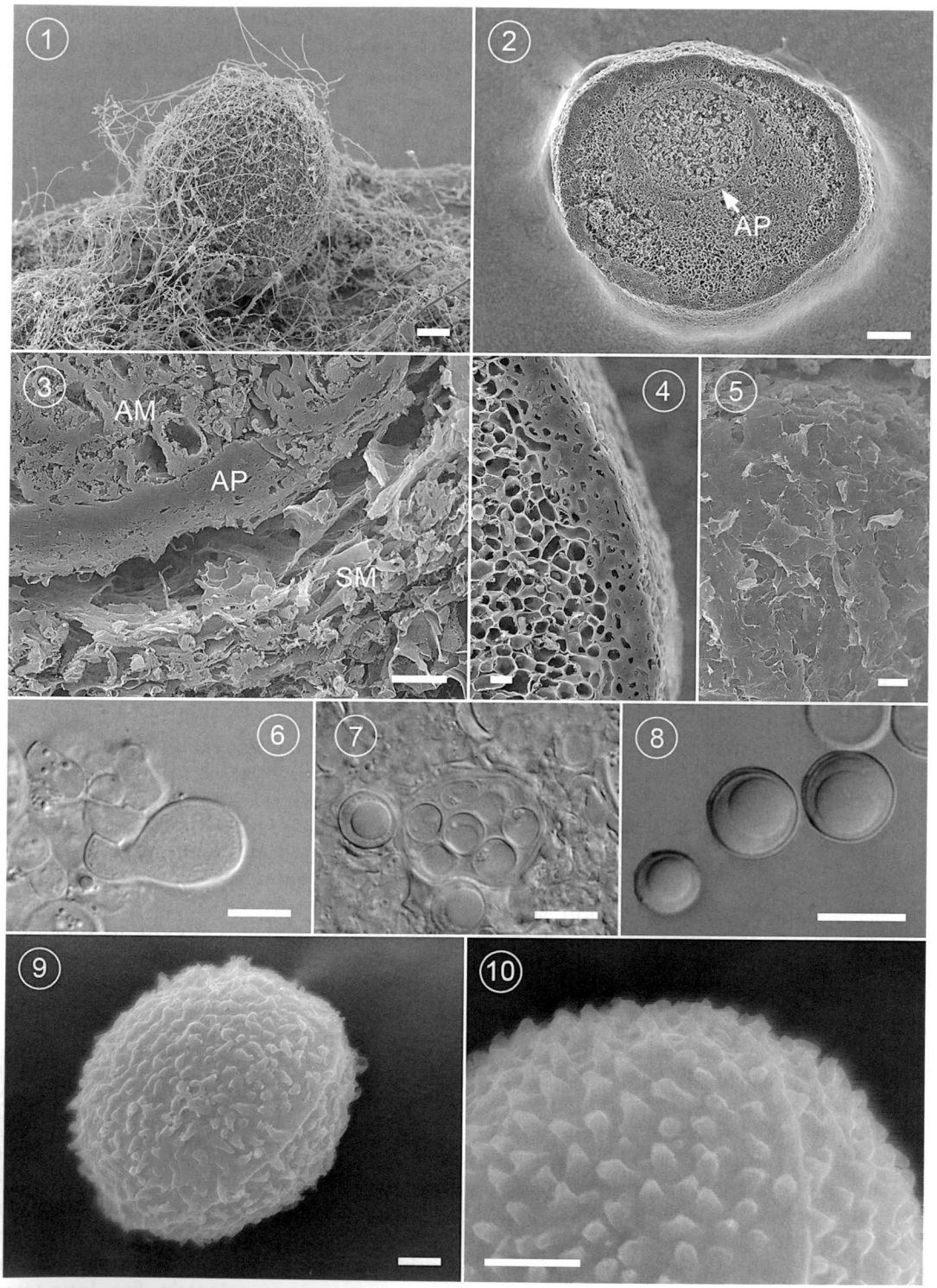
Stromata were fixed, critical point dried, sputter coated and examined with a JEOL JSM-5900LV scanning electron microscope according to Horn et al (2009).

TAXONOMY

Petromyces parasiticus B.W. Horn, I. Carbone et Ramirez-Prado, sp. nov. FIGS. 1–10

Coloniae in agaro Czapekii crescentes post 7 dies in temperatura 25 C diametro 3.0–4.5 cm atque in temperatura 37 C 4.0–6.5 cm attingentes, velutinae vel leniter funiculosae, atro-virides. Sclerotia stromataque globosa vel ellipsoidalia, 300–1200 µm, atro-brunnea vel atra, contextum pseudoparenchymaticum continentia. Ascocarpi 1–13, intra stromata geniti, globosi vel subglobosi vel irregulariter formati, maturitate 160–530 µm, non ostiolati, peridio 6.5–18.7 µm crasso circumdati. Asci globosi vel subglobosi, 19–29 × 16–27 µm, plerumque ascosporas 8 continentes. Ascosporae ubique oblatae, sed in aspectu frontali globosae vel subglobosae, 7.1–13.0 × 6.5–12.0 µm, leniter tuberculatae, crista tenui aequatoriali praeditae, hyalinae vel pallide brunneae, gutta olei unica magna continentes. Capitula conidialia uniseriata vel interdum biseriata, laxe radiata, diametro usque ad 450 µm. Stipites proxime sub vesicula 5–11 µm lati, longitudine 200–600 µm, hyalini, echinulati vel sublaeves. Vesiculae globosae vel subglobosae, 12–35 µm diametro. Metulae 7.0–10.0 × 3.5–7.0 µm. Phialides 7.0–10.0 × 2.5–5.0 µm. Conidia globosa vel subglobosa, 4.0–6.0 µm, distincte asperata.

Colonies on Czapek agar attaining 3.0–4.5 cm diam in 7 d at 25 C; growth at 37 C in 7 d reaching 4.0–6.5 cm diam. Colony surface velvety to weakly funiculose, consisting of abundant conidial heads. Conidial heads en masse dark green (27–29F4–5; Kornerup and Wanscher 1978) at 14 d. Reverse light yellow brown. *Sclerotia* (FIG. 1) and *stromata* similar in external appearance, globose to ellipsoidal, (250–)300–1200(–1300) µm, white becoming pink brown and finally dark brown to black; inner matrix light to dark brown, consisting of pseudoparenchymatous tissue (FIG. 4). *Ascocarps* (FIG. 2) produced within stromata, globose to subglobose or irregularly shaped, nonostiolate, with white to light brown interior; each stroma containing 1–3(–4) fertile ascocarps, 1–13(–15) infertile ascocarps or a combination of the two; *fertile ascocarps* (130–)160–530(–550) µm (mean = 313 ± 105 µm, n = 73) × (100–)140–420(–470) µm (mean = 249 ± 80 µm); *infertile ascocarps* often discoid, (40–)50–230(–280) µm (mean = 122 ± 41 µm, n = 315) × (40–)50–200(–230) µm (mean = 107 ± 34 µm); *ascocarp peridium* (FIG. 3) 6.5–18.7 µm thick, yellow brown to red brown, consisting of compact layers of irregular flattened cells (FIG. 5). *Asci* (FIGS. 6, 7) globose to subglobose, often con-



FIGS. 1–10. *Petromyces parasiticus.* 1. Sclerotium formed in culture. 2. Sectioned stroma showing single ascocarp. 3. Cross section of ascocarp peridium with stromal and ascocarp matrices on each side. 4. Section of stroma showing outer peridium and pseudoparenchymatous tissue of matrix. 5. Surface view of ascocarp peridium showing irregular flattened cells. 6. Immature ascus. 7. Ascus with ascospores embedded in ascocarp matrix; two free ascospores are present near the ascus. 8. Ascospores containing single oil droplets. 9, 10. Ascospore showing finely tuberculate ornamentation and an equatorial ridge. 1–5, 9, 10 = SEM; 6–8 = differential interference contrast microscopy. Bars 1, 2 = 100 μm; 4–8 = 10 μm; 9, 10 = 1 μm. Abbreviations: SP, stromal peridium; SM, stromal matrix; AP, ascocarp peridium; AM, ascocarp matrix.

taining eight ascospores but irregular numbers (1–6) not uncommon, (17.0–)19.0–29.0(–32.0) μm (mean = 23.3 ± 3.5 μm, n = 64) × (15.5–)16.0–27.0(–28.0) μm (mean = 20.9 ± 3.1 μm). *Ascospores* (FIGS. 8–10) oblate, finely tuberculate with a thin equatorial ridge, hyaline to pale brown, generally containing a single large oil droplet, globose to subglobose in face view, variable in size, (6.0–)7.1–13.0(–15.0) μm (mean = 9.7 ± 1.5 μm, n = 94) × (6.0–)6.5–12.0(–13.9) μm (mean = 9.0 ± 1.5 μm). *Conidial heads* uniseriate or occasionally biseriate, loosely radiate, up to 450 μm diam. *Stipes* 200–600(–900) μm long, 5.0–11.0 μm wide immediately below vesicle, hyaline, echinulate to nearly smooth. *Vesicles* globose to subglobose, 12–35 μm diam. *Metulae* 7.0–10.0 × 3.5–7.0 μm. *Phialides* 7.0–10.0 × 2.5–5.0 μm. *Conidia* globose to subglobose, 4.0–6.0(–7.0) μm, distinctly roughened.

HOLOTYPE. Dried slant culture with ascocarp-bearing stromata consisting of *A. parasiticus* NRRL 29538 (*MAT1-1*) crossed with *A. parasiticus* NRRL 29570 (*MAT1-2*); deposited with the National Fungus Collections, US Department of Agriculture, Beltsville, Maryland (BPI 878821). NRRL 29538 and NRRL 29570 were isolated from soil collected 8 Jun 1992 in a peanut field, Terrell County, Georgia, USA (Horn and Greene 1995). Living cultures of both strains have been deposited in the ARS Culture Collection, Peoria, Illinois, USA.

Additional sexual crosses examined: All *A. parasiticus* strains have the same provenience as the holotype; fertility of crosses was reported by Horn et al (2009). Additional crosses (in order of *MAT1-1* × *MAT1-2*) were NRRL 29581 × 29604; NRRL 29586 × 29604; NRRL 29590 × 29570; NRRL 29612 × 29570; NRRL 29590 × 29606; NRRL 29538 × 29604; NRRL 29607 × 29603; NRRL 29612 × 29604; NRRL 29612 × 29606.

DISCUSSION

Section *Flavi* of *Aspergillus* comprises a monophyletic assemblage of both aflatoxigenic and nonaflatoxigenic species (Peterson 2008). The section contains *A. alliaceus*, a species that originally was placed in the "*Aspergillus wentii* group" (Thom and Raper 1945, Kozakiewicz 1989) or the "*Aspergillus ochraceus* group" (Raper and Fennell 1965) due primarily to its yellow conidial heads but subsequently was shown to belong instead to section *Flavi* based on rDNA sequence analysis (Peterson 1995). The morphology of the sexual state of *A. alliaceus* first was described by Fennell and Warcup (1959) and a new genus, *Petromyces* Malloch & Cain (1972), subsequently was erected to accommodate this single species. *Petromyces* is characterized by the formation of multiple, nonostiolate ascocarps within the pseudoparenchymatous matrix of stromata and by the formation of conidia on vesiculate conidiophores (*Aspergillus* anamorphic state). Because *A. alliaceus* and *A. parasiticus* are closely related phylogenetically (Peterson 2008) it is not surprising that the sexual state of *A. parasiticus* also falls within the generic concept of *Petromyces*.

The sexual states of *P. parasiticus* and *P. alliaceus* differ in several respects. Ascospores of *P. parasiticus* are finely tuberculate and 7.1–13 × 6.5–12 μm, whereas those of *P. alliaceus* are smooth and 5.5–9 × 5–7 μm (Fennell and Warcup 1959). Ascospore ornamentation in *P. parasiticus* is faintly visible under the light microscope and is most distinctive when viewed with SEM. Both species have equatorial lines on their ascospores. Under SEM the equatorial line on ascospores of *P. parasiticus* is clearly seen as a low ridge instead of the furrow described for ascospores of *P. alliaceus* (Fennell and Warcup 1959, Malloch and Cain 1972). However ascospore morphology in *P. alliaceus* was based on light microscopy. SEM micrographs of ascospores of *P. albertensis* Tewari (1985), a species considered to be synonymous with *P. alliaceus* (Frisvad and Samson 2000, Varga et al 2000, McAlpin and Wicklow 2005b), also show an equatorial ridge.

The anamorphic states of *P. parasiticus* and *P. alliaceus* are easily distinguished. Conidial heads of *P. parasiticus* are dark green and predominantly uniseriate (Klich and Pitt 1988), as opposed to the conidial heads of *P. alliaceus*, which range from yellow orange to cinnamon buff and are mostly biseriate (Raper and Fennell 1965). In *P. parasiticus* stipes are commonly echinulate and 200–600 μm long; vesicles are 12–35 μm diam; and conidia are distinctly roughened, globose to subglobose, and 4–6 μm diam. In *P. alliaceus* stipes are smooth and up to 2 mm long; vesicles are mostly 40–60 μm diam; and conidia are smooth, subglobose to oval and 2.5–4 × 2–3.5 μm (Raper and Fennell 1965).

In contrast to the heterothallic condition of *P. parasiticus*, *P. alliaceus* is homothallic (Fennell and Warcup 1959, McAlpin and Wicklow 2005a) due to the presence of tightly linked *MAT1-1* and *MAT1-2* genes (Ramirez-Prado et al 2008). McAlpin and Wicklow (2005a) reported that approximately 25–28% of *P. alliaceus* stromata produce ascocarps on mixed cereal agar and speculated that the infertile stromata act as sclerotia for long-term survival. Sclerotium formation in heterothallic *P. parasiticus* commonly occurs in individuals representing a single mating type (Horn and Greene 1995) and is not induced by sexual mating. Therefore such structures appear to serve a dual purpose in the life cycle of *P. parasiticus*: (i) withstanding adverse environmental conditions in individual unmated strains (sclerotia) and (ii) providing for genetic recombination in the

formation of ascospores after mating of sexually compatible strains (stromata). Sexual development has not been studied extensively in *Petromyces*. Fennell and Warcup (1959) reported evidence of "channeling" of the stromatic tissue before ascocarp formation in *P. alliaceus*. In an electron microscope study of this species various regions of the stroma underwent autolysis and the autolyzed cells were colonized by proliferating hyphae (Tewari 1983). It is not known when mating occurs in relation to stroma development.

Crosses between strains of the opposite mating type in *P. parasiticus* vary greatly in the degree of fertility, with some crosses not producing ascocarps, other crosses forming small numbers of infertile ascocarps and still others frequently producing fertile ascospore-bearing ascocarps (Horn et al 2009). Infertile ascocarps did not produce asci or ascospores and did not show further development with additional culture incubation. Various prezygotic and postzygotic genetic barriers in fungi prevent completion of the sexual cycle despite the compatibility of mating types (Anderson et al 1992) and might account for the differences in *P. parasiticus* fertility. In addition infertile ascocarps often were accompanied by larger fertile ascocarps, where they frequently were appressed and flattened against the peridium of the stroma and/or fertile ascocarp. In instances of multiple ascocarps developing within a single stroma, competition for nutrients or space might limit the number of fertile ascocarps and result in abortion of the other ascocarps.

Petromyces parasiticus and *P. alliaceus* may not be the only species within section *Flavi* capable of sexual reproduction. The aflatoxin-producing species *A. flavus*, *A. nomius* and *A. pseudotamarii* Y. Ito et al and the aflatoxin-nonproducing species *A. caelatus* B.W. Horn and *A. leporis* States & M. Chr. all commonly produce sclerotia (States and Christensen 1966, Kurtzman et al 1987, Horn et al 1996, Horn 1997, Ito et al 2001). Additional research might reveal that sexual reproduction is the rule rather than the exception in section *Flavi*.

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LITERATURE CITED

Anderson JB, Kohn LM, Leslie JF. 1992. Genetic mechanisms in fungal adaptation. In: Carroll GC, Wicklow DT, eds. The fungal community: its organization and role in the ecosystem. New York: Marcel Dekker. p 73–98.

Asplin FD, Carnaghan RBA. 1961. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Vet Rec 73: 1215–1219.

Barros GG, Chiotta ML, Reynoso MM, Torres AM, Chulze SN. 2007. Molecular characterization of *Aspergillus* section *Flavi* isolates collected from peanut fields in Argentina using AFLPs. J Appl Microbiol 103:900–909.

Carbone I, Jakobek JL, Ramirez-Prado JH, Horn BW. 2007. Recombination, balancing selection and adaptive evolution in the aflatoxin gene cluster of *Aspergillus parasiticus*. Molec Ecol 16:4401–4417.

Cole RJ. 1986. Etiology of turkey "X" disease in retrospect: a case for the involvement of cyclopiazonic acid. Mycotoxin Res 2:3–7.

Cullen JM, Newberne PM. 1994. Acute hepatotoxicity of aflatoxins. In: Eaton DL, Groopman JD, eds. The toxicology of aflatoxins: human health, veterinary, and agricultural significance. San Diego: Academic Press. p 3–26.

Eaton DL, Groopman JD, eds. 1994. The toxicology of aflatoxins: human health, veterinary, and agricultural significance. San Diego: Academic Press. 544 p.

Fennell DI, Warcup JH. 1959. The ascocarps of *Aspergillus alliaceus*. Mycologia 51:409–415.

Frisvad JC, Samson RA. 2000. *Neopetromyces* gen. nov. and an overview of teleomorphs of *Aspergillus* subgenus *Circumdati*. Stud Mycol 45:201–207.

Geiser DM, Pitt JI, Taylor JW. 1998. Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*. Proc Nat Acad Sci USA 95:388–393.

———, Timberlake WE, Arnold ML. 1996. Loss of meiosis in *Aspergillus*. Mol Biol Evol 13:809–817.

Horn BW. 1997. *Aspergillus caelatus*, a new species in section *Flavi*. Mycotaxon 61:185–191.

———. 2007. Biodiversity of *Aspergillus* section *Flavi* in the United States: a review. Food Addit Contam 24:1088–1101.

———, Greene RL. 1995. Vegetative compatibility within populations of *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii* from a peanut field. Mycologia 87:324–332.

———, ———, Sobolev VS, Dorner JW, Powell JH, Layton RC. 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii*. Mycologia 88:574–587.

———, Ramirez-Prado JH, Carbone I. 2009. Sexual reproduction and recombination in the aflatoxin-producing fungus *Aspergillus parasiticus*. Fungal Genet Biol 46: 169–175.

Ito Y, Peterson SW, Wicklow DT, Goto T. 2001. *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *Flavi*. Mycol Res 105:233–239.

Klich MA, Pitt JI. 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans Brit Mycol Soc 91:99–108.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Eyre Methuen. 252 p.

Kozakiewicz Z. 1989. *Aspergillus* species on stored products. Wallingford, United Kingdom: CAB International. 188 p.

Kurtzman CP, Horn BW, Hesseltine CW. 1987. *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie van Leeuwenhoek 53:147–158.

Malloch D, Cain RF. 1972. The Trichocomataceae: ascomycetes with *Aspergillus*, *Paecilomyces*, and *Penicillium* imperfect states. Can J Bot 50:2613–2628.

McAlpin CE, Wicklow DT. 2005a. Culture media and sources of nitrogen promoting the formation of stromata and ascocarps in *Petromyces alliaceus* (*Aspergillus* section *Flavi*). Can J Microbiol 51:765–771.

———, ———. 2005b. DNA fingerprinting analysis of *Petromyces alliaceus* (*Aspergillus* section *Flavi*). Can J Microbiol 51:1039–1044.

———, ———, Platis CE. 1998. Genotypic diversity of *Aspergillus parasiticus* in an Illinois corn field. Plant Dis 82:1132–1136.

Peterson SW. 1995. Phylogenetic analysis of *Aspergillus* sections *Cremei* and *Wentii*, based on ribosomal DNA sequences. Mycol Res 99:1349–1355.

———. 2008. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 100: 205–226.

———, Ito Y, Horn BW, Goto T. 2001. *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*. Mycologia 93:689–703.

Ramirez-Prado JH, Moore GG, Horn BW, Carbone I. 2008. Characterization and population analysis of the mating-type genes in *Aspergillus flavus* and *A. parasiticus*. Fungal Genet Biol 45:1292–1299.

Raper KB, Fennell DI. 1965. The genus *Aspergillus*. Baltimore: Williams & Wilkins. 686 p.

Sargeant K, Sheridan A, O'Kelly J. 1961. Toxicity associated with certain samples of groundnuts. Nature 192:1096–1097.

Speare AT. 1912. Fungi parasitic upon insects injurious to sugar cane. Pathology and Physiological Series, Bulletin No. 12. Honolulu: Hawaiian Sugar Planters' Association Experiment Station. 62 p.

States JS, Christensen M. 1966. *Aspergillus leporis*, a new species related to *A. flavus*. Mycologia 58:738–742.

Tewari JP. 1983. Stromatic cell autolysis in *Petromyces alliaceus* during ascocarp formation. Trans Br Mycol Soc 80:127–130.

———. 1985. A new indeterminate stromatal type in *Petromyces*. Mycologia 77:114–120.

Thom C, Raper KB. 1945. A manual of the aspergilli. Baltimore: Williams & Wilkins. 373 p.

Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. 2003. Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ Health Perspect 111:217–220.

van Edmond HP, Jonker MA. 2005. Worldwide regulations on aflatoxins. In: Abbas HK, ed. Aflatoxin and food safety. Boca Raton, Florida: Taylor & Francis. p 77–93.

Varga J, Tóth B, Kevei E, Palágyi A, Kozakiewicz Z. 2000. Analysis of genetic variability within the genus *Petromyces*. Antonie van Leeuwenhoek 77:83–89.